

CLAIMS

1. A method for selectively separating and purifying RNA from a mixture solution of nucleic acid containing DNA and RNA,

5 the method using a cartridge for separation and purification of nucleic acid comprising a container having at least two openings, and the container receives a nucleic acid-adsorbing porous membrane which a solution can pass through,

wherein the method comprising the steps of:

(1-a) adsorbing nucleic acid to the nucleic acid-adsorbing porous membrane;

10 (1-b) washing the nucleic acid-adsorbing porous membrane by a washing solution, while the nucleic acid is adsorbed to the nucleic acid-adsorbing porous membrane;

(1-c) subjecting the nucleic acid-adsorbing porous membrane to a DNase treatment;

(1-d) washing the nucleic acid-adsorbing porous membrane with the washing solution; and

15 (1-e) desorbing the RNA from the nucleic acid-adsorbing porous membrane by a recovering solution, so as to discharge the recovering solution out of the cartridge,

wherein in the step (1-c), a total amount of a DNase solution is 130 μ l or less per 1 cm^2 of the nucleic acid-adsorbing porous membrane.

2. The method for selectively separating and purifying RNA according to claim 1,

wherein the DNase solution has a DNase concentration of 10 to 10000 Kunitz U/mL.

20

3. The method for selectively separating and purifying RNA according to claim 1 or 2,

wherein the nucleic acid adsorbing porous membrane comprises an organic polymer to which the nucleic acid is adsorbed by a weak interaction involving substantially no ionic bond.

25

4. The method for selectively separating and purifying RNA according to claim 3,

wherein the nucleic acid-adsorbing porous membrane has a hydroxyl group.

30

5. The method for selectively separating and purifying RNA according to any of claims 1 to 4,

wherein the nucleic acid adsorbing porous membrane comprises an organic material obtained

by saponification of a mixture of acetyl celluloses different from each other in acetyl value.

6. The method for selectively separating and purifying RNA according to any of claims 1 to 5,

wherein the nucleic acid adsorbing porous membrane has a front area and a back area

asymmetrical with each other.

7. The method for selectively separating and purifying RNA according to any of claims 1 to 6, wherein the mixture solution of nucleic acid is a solution where a water-soluble organic solvent is further added to a mixed solution obtained by mixing a nucleic acid solubilizing reagent added to a test sample with the test sample.

8. The method for selectively separating and purifying RNA according to claim 7, wherein the test sample is a cultured cell.

10 9. The method for selectively separating and purifying RNA according to claim 8, wherein the cultured cell is a cell grown in a suspension.

10. The method for selectively separating and purifying RNA according to claim 8, wherein the cultured cell is a cell grown in a monolayer.

15 11. The method for selectively separating and purifying RNA according to claim 7, wherein the test sample is an animal tissue.

12 The method for selectively separating and purifying RNA according to any of claims 7 to 20 11, wherein the test sample is homogenized before or after adding nucleic-acid solubilizing reagent.

13. The method for selectively separating and purifying RNA according to any of claims 7 to 12, wherein the nucleic acid-solubilizing reagent comprises at least one of a chaotropic salt, a 25 nucleic acid stabilizing agent, a surfactant, a buffer and a defoaming agent.

14. The method for selectively separating and purifying RNA according to claim 13, wherein the chaotropic salt is at least one of a guanidine hydrochloride and a guanidine thiocyanate.

30 15. The method for selectively separating and purifying RNA according to any of claims 7 to 14, wherein the water-soluble organic solvent comprises at least one of methanol, ethanol, propanol and an isomer thereof, and butanol and an isomer thereof.

16. The method for selectively separating and purifying RNA according to any of claims 1 to
15,

5 wherein the washing solution is a solution containing at least one alcohol selected from
methanol, ethanol, propanol and an isomer thereof, and butanol and an isomer thereof,

wherein the washing solution contains said at least one alcohol in an amount of 1 to 100% by
weight.

17. The method for selectively separating and purifying RNA according to any of claims 1 to
10 16,

 wherein the recovering solution is a solution having a salt concentration of 0.5 mol/L or less.

18. The method for selectively separating and purifying RNA according to any of claims 1 to
15 17,

 wherein a pressure difference-generating apparatus is a pump detachably connected to one
opening of the cartridge for separation and purification of nucleic acid.

19. A kit of a cartridge for separation and purification of nucleic acid and a reagent for
20 performing a method according to any of claims 1 to 18.

20. An apparatus for automatically performing a method according to any of claims 1 to 18.

21. An apparatus for automatically using a kit according to claim 19.

25 22. A method for selectively separating and purifying RNA or DNA, which comprises the
steps of:

 (2-a) adsorbing nucleic acid to a nucleic acid-adsorbing porous membrane by passing a mixture
solution of nucleic acid containing RNA and DNA through the nucleic acid-adsorbing porous
membrane;

30 (2-b) washing the nucleic acid-adsorbing porous membrane by passing a washing solution
through the nucleic acid-adsorbing porous membrane, while the nucleic acid is adsorbed to the
nucleic acid-adsorbing porous membrane; and

 (2-c) desorbing the nucleic acid from the nucleic acid-adsorbing porous membrane by passing a
recovering solution through the nucleic acid-adsorbing porous membrane,

wherein the washing solution contains a water-soluble organic solvent having a concentration of 50% by weight or less, and the washing solution does not contain a chaotropic salt.

23. The method for selectively separating and purifying RNA or DNA according to claim 22,
5 wherein the washing solution contains a water-soluble organic solvent having a concentration of 5 to 40% by weight.

24. The method for selectively separating and purifying RNA or DNA according to claim 22 or
23,

10 wherein the nucleic acid-adsorbing porous membrane is a porous membrane comprising an organic polymer to which the nucleic acid is adsorbed by a weak interaction involving substantially no ionic bond.

25. The method for selectively separating and purifying RNA or DNA according to any of
15 claims 22 to 24,

wherein the nucleic acid-adsorbing porous membrane is a porous membrane comprising an organic polymer having a hydroxyl group.

26. The method for selectively separating and purifying RNA or DNA according to any of
20 claims 22 to 25,

wherein the nucleic acid-adsorbing porous membrane is a porous membrane comprising an organic material obtained by saponification of a mixture of acetyl celluloses different from each other in acetyl value.

25 27. The method for selectively separating and purifying RNA or DNA according to any of
claims 22 to 26,

wherein the nucleic acid-adsorbing porous membrane has a front area and a back area
asymmetrical with each other.

30 28. The method for selectively separating and purifying RNA or DNA according to any of
claims 22 to 27,

wherein the sample solution is a solution where a water-soluble organic solvent is added to a solution obtained by treating a cell or virus-containing test sample with a nucleic acid-solubilizing reagent.

29. The method for selectively separating and purifying RNA or DNA according to claim 28,
wherein the test sample is a cultured cell.

5 30. The method for selectively separating and purifying RNA or DNA according to claim 28,
wherein the test sample is an animal tissue.

31. The method for selectively separating and purifying RNA according to any of claims 28 to
30,

10 wherein the test sample is homogenized before or after adding nucleic-acid solubilizing reagent.

32. The method for selectively separating and purifying RNA or DNA according to claim 28,
wherein the nucleic acid-solubilizing reagent comprises at least one of a chaotropic salt, a
nucleic acid stabilizing agent, a surfactant, a buffer and a defoaming agent.

15 33. The method for selectively separating and purifying RNA or DNA according to claim 32,
wherein the chaotropic salt is at least one of a guanidine hydrochloride and a guanidine
thiocyanate.

20 34. The method for selectively separating and purifying RNA or DNA according to any of
claims 22 to 33,
wherein the water-soluble organic solvent is at least one alcohol selected from methanol,
ethanol, propanol and an isomer thereof, and butanol and an isomer thereof.

25 35. The method for selectively separating and purifying RNA or DNA according to any of
claims 22 to 34,
wherein the washing solution is a solution containing at least one alcohol selected from
methanol, ethanol, propanol and an isomer thereof, and butanol and an isomer thereof in an amount of
5 to 50% by weight.

30 36. The method for selectively separating and purifying RNA according to any of claims 22 to
35,
wherein the washing solution contains water-soluble salt.

37. The method for selectively separating and purifying RNA according to claim 36, wherein the concentration of water-soluble salt is 10mmol/L or more.

5 38. The method for selectively separating and purifying RNA according to any of claim 36 or 37,

wherein the concentration of water-soluble salt is in a range of 10mmol/L to 1mol/L.

9 39. The method for selectively separating and purifying RNA or DNA according to any of claims 22 to 38,

10 wherein the washing solution is a solution containing a chloride in an amount of 10 mmol/L to 1 mol/L.

15 40. The method for selectively separating and purifying RNA or DNA according to any of claims 22 to 39,

15 wherein the recovering solution is a solution capable of desorbing an adsorbed RNA from the nucleic acid-adsorbing porous membrane having a salt concentration of 0.5 mol/L or less.

20 41. The method for selectively separating and purifying RNA or DNA according to any of claims 22 to 40,

20 wherein in each of the steps of (2-a), (2-b) and (2-c), the sample solution containing the nucleic acid, the washing solution and the eluting solution are passed through the nucleic acid-adsorbing porous membrane by using (i) a cartridge for separation and purification of nucleic acid comprising a container having at least two openings, and the cartridge for separation and purification of nucleic acid receives the nucleic acid-adsorbing porous membrane which a solution can pass through in the 25 container and (ii) a pressure difference-generating apparatus,

wherein the pressure difference-generating apparatus is a pump detachably connected to one opening of the cartridge for separation and purification of nucleic acid.

30 42. A kit of a cartridge for separation and purification of nucleic acid and a reagent for performing a method according to any of claims 22 to 41.

43. An apparatus for automatically performing a method according to any of claims 22 to 41.